

EFFECT OF CALOTROPIS GIGANTEA FLOWERS EXTRACTS ON MAST CELL DEGRANULATION IN RATS.

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Summary

Calotropis gigantea Linn. commonly known as milkweed or swallow-wort found as wasteland weed. *Calotropis gigantea* Linn. distributed in tropical and sub-tropical Asia and Africa. A tall shrub reaching 2-3 m high used in medicine in various forms, such as taila, tincture etc. A taila made by boiling together Sesamum oil and *Calotropis* flowers juice and turmeric is useful in eczema and eruptive skin diseases. Dried flowering are given every morning as a remedy for asthma. Fine powder of root-bark prescribed in cases of syphilis, and fever. Powders of the dried leaves dusted upon wounds to destroy excessive granulation and promote healing action. The tincture from the leaves tried as an antiperiodic in cases of intermittent fevers. Plant has been investigated for much of therapeutic responses and as well a pilot study on the clinical efficacy of *Calotropis gigantea* dried flowers shown significant improvement in some respiratory diseases. The present study aimed at investigating the anti-anaphylactic property of petroleum ether, ethanol (95%), water extract of flowers of *Calotropis gigantea*, obtained by successive extraction in specific *in-vivo* animal models. Compared to petroleum ether and water extract ethanolic (95%) extract (CGEE) shown significant results ($P < 0.05$). Mast cells were protected at a dose of 400 & 600 mg/kg, p.o. by 72.25 % & 77.14 % respectively by CGEE as compared to standard drug DSCG (82.49%). Phytochemical screening shown presence of flavonoids, carbohydrates as a reducing sugar and anthocyanins. The results obtained suggests that the ethanol extract of *Calotropis gigantea* flowers possess antihistaminic, mast cell stabilizing effect and hence confirms its potential role in the treatment of anaphylaxis and allergic disorders as claimed in literature.

Key Words: *Calotropis gigantea*, antihistaminic, mast cell degranulation.

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Introduction

Anaphylaxis is an acute, potentially life-threatening syndrome, with multisystemic manifestations resulting from the rapid release of inflammatory mediators. Anaphylaxis is an important clinical problem associated with morbidity, mortality, and increased health care costs (1, 2). Anaphylaxis is an antigen-antibody reaction in which antigen is called anaphylactogen and the antibody-anaphylactin (3). Generally, more rapid onset of symptoms after exposure to the offending agent, the more severe the reaction (4). Various drugs were in use in modern medicines for the treatment of immediate type of hypersensitivity reaction but after effects and side effect of these are quite disturbing. Availability of plethora of natural medicines with proven clinical efficacy are the main focus in development of new drug from natural sources with more therapeutic efficacy and safety. One of such a plant in the indigenous system of medicine *Calotropis gigantea* common wasteland weed widely distributed in tropical and sub-tropical Asia and Africa (5, 6) having claimed therapeutic potential in treatment of allergic conditions. The plant is a popular remedy for snake-bite and scorpion-sting. A proteolytic enzyme, somewhat similar to papain, has been found in the milky juice of flowers and acting like digitalis on the heart (7, 8). The flower were described in ancients Ayurveda as sweet-bitter, anthelmintic, analgesic, astringent, cures inflammations, tumors, kapha, rat-bite. The flowers are considered as digestive, stomachic, tonic, useful in asthma, catarrh and loss of appetite (8). Aerial parts of *Calotropis gigantea* reported for anti- diarrheal activity (9). Latex of *Calotropis gigantea* evaluated for procoagulating activity associated with fibrinolytic activity (10). Alcoholic extract of the dried peeled roots of *Calotropis gigantea* posses CNS activity (11). Alcoholic extract of the flowers of *Calotropis gigantea* reported for analgesic activity (12). Alcoholic extract of roots evaluated for pregnancy intraceptive activity (13). Aerial parts of total aqueous extract and water soluble fraction of *Calotropis gigantea* were evaluated for immunomodulatory, anti-inflammatory, anticancer and antimitotic activity (14, 15, 16). Alcoholic extract of stems posses hepatoprotective activity (17). Regardless much of these investigations the flowers utility in allergic condition were aimed in this present investigation to validate the traditional claims of flowers in allergy and anaphylaxis. As the drug is highly valued in “Ayurvedic system of medication” for allergy and other inflammatory disorders so it was thought worthy to evaluate this drug for its anti-anaphylactic aspects using different models.

Methods

Collection of plant material

The fresh flowers of *Calotropis gigantea* were collected in the month of August 2009 at Chopda Dist. Jalgaon, MS, India. The plant and flower material authenticated by Dr. Chakrawarty, Botanical Survey of India, Pune. A voucher specimen (No. ANUACG1) was deposited in the Botanical Survey of India, Pune, India.

Extraction of plant material

Calotropis gigantea flowers were shed dried and reduced to coarse powder. The Coarse powdered flowers (1000 g) were subjected for successive extraction using Soxhlet assembly, initially with petroleum ether (60⁰-80⁰), then the marc dried in open air and further subjected for ethanolic extraction (95%), finally the same marc after drying rendered for extraction using purified water. Extracts was filtered and concentrated by evaporation under reduced pressure with yield of (CGPEE) petroleum ether extract 1.24% w/w/, (CGEE) ethanol (95%) 11.66% w/w and (CGWE) water extract 10.45% w/w. Preliminary phytochemical screening showed presence of flavonoids, carbohydrates as a reducing sugar and anthocyanins.

Animals

Male and female albino mice weighing 100-200 gm were housed under standard laboratory condition in a group of five (n=6). Animals had free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethical committee (IAEC) has approved the protocol of the study.

Chemicals and reagents

Disodium cromoglycate Cipla, India; Compound 48/80 Unichem, India; RPMI Buffer medium 1640 of Hi Media has been purchased. Toluidine blue was purchased from Research Lab. India.

Acute toxicity studies

Healthy adult albino rats (100- 200g) used for acute toxicity studies as per guidelines (OECD 423) suggested by the organization for economic co-operation and development (OECD-2001). The rats were observed continuously for 2 h for behavioral and autonomic profiles and for any sign of toxicity or mortality up to a period of seven days (OECD Guideline For The Testing of Chemicals: Guidance document on acute oral toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment 2000). Highest dose at which no toxic signs are seen, one fifth of that should be taken as effective dose.

In vivo studies

Mast cell degranulation (18, 19)

Rats were divided in five groups, six animals in each group. The seven days drug treatment schedules were followed. Group I received vehicle (5 % PEG-400, 5ml / kg, p.o.) Group- II treated with standard drug disodium cromoglycate (DSCG, 200µg/kg, i.p.). Group-III, IV and V were treated with extract 200, 400 and 600 mg/kg, p.o. respectively. On day seventh, each animal was injected with 4 ml/kg, 0.9 % saline solution, into peritoneal cavity. By gentle massage, peritoneal fluid was collected after 5 mins and transferred into siliconised test tubes containing 7-10 ml RPMI-1640 buffer medium (pH 7.2-7.4). This solution was then centrifuged at 400-500 RPM. Pellet of mast cell was washed with same buffer medium twice by centrifugation, discarding supernatant. Mast cell suspension (1x10⁶ cells/ml) were challenged with compound 48/80 (0.5 µg/ml), incubated at 37°C in a water bath for 10 mins. Followed by staining with 10% toluidine blue and observed under microscope (10X). Total 100 cells were counted

from different visual area. Percent protection against degranulation was calculated using method described by Lakdawala (18).

Statistical analysis

The data is presented as mean±SEM. The statistical significance between the groups has been tested by ANOVA followed by Dunnett’s test. A probability value less than 0.05 were considered as significant.

Results

Effect of Pet ether (CGPEE), Ethanol (CGEE) and Water extract (CGWE) on mast cell degranulation.

Clonidine challenge resulted in significant degranulation of mast cell. Pretreatment of sensitized animal with standard drug DSCG shown protection 82.49% and CGEE at a dose of (400& 600 mg/kg, p.o.) shown percentage protection of 72.25 % & 77.14 % respectively as seen in figure 2. Petroleum ether and water extract of flowers was unable to show the significance.

Table 1. Effect of CGEE on Mast cells degranulation in rats

Group	Treatment	Dose	No. of Mast cell degranulation
I	Control (5 % PEG-400)	5ml/kg, p.o.	82.25±1.712
II	DSCG	200µg/ kg, i.p	14.40±1.242
III	CGEE	200mg/kg, p.o.	27.60±2.252
IV	CGEE	400mg/kg, p.o.	22.82±1.126*
V	CGEE	600mg/kg, p.o.	18.80±1.698*

n=6, values are expressed in mean±SEM; **Control** = Vehicle (5 % PEG-400, 5ml / kg, p.o.) *p< 0.05 compared with control group (ANOVA followed by Dunnett’s test)

Statistically significant data CGEE – *Calotropis gigantea* flower ethanol extract

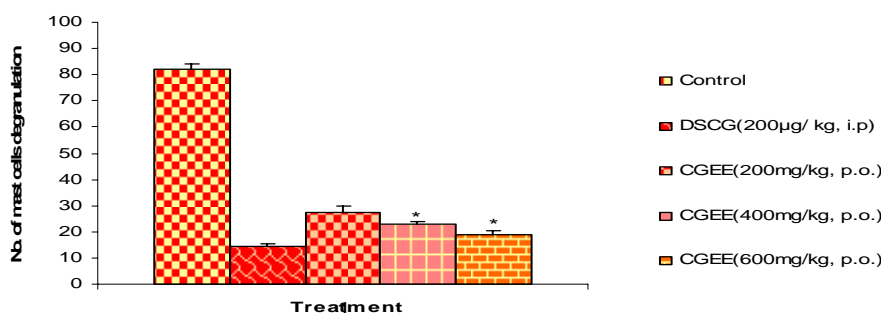


Figure 2 Effect of CGEE on Mast cells degranulation in rats

Discussion

Anaphylaxis is an immediate hypersensitivity multisystemic manifestations resulting from the rapid release of inflammatory mediators. Histamine is an important mediator of immediate allergic (type-1) and inflammatory reactions followed by release and degranulation of mast cell. Mast cells play a critical role in immediate hypersensitivity and allergic reactions when activated through immunoglobulin E (IgE) by specific antigens. Degranulation of mast cells is an important in the initiation of immediate responses following exposure to allergens (20). Once binding of allergen to cell-bound IgE occurs, mediators such as histamine, eosinophils, neutrophils, chemotactic factor LTC₄, LTD₄, and LTE₄, PG, and PAF etc. are released from mast cells. Mast cell degranulation believed to be an integral cause of exercise-induced bronchospasm following either drying or cooling of the airways and hyper sensitivity reactions (21). Controlling the release or offering the protection against degranulation of mast cells is phenomenon of choice. Present study shows statistically significant stabilization of mast cell by CGEE at a dose of (400 & 600 mg/kg, p.o.) shown percentage protection of 72.25 % & 77.14 % respectively as compared to standard drug DSCG (82.49%). This may be by raising the level of cyclic adenosine monophosphate which further relax airway smooth muscle and inhibit the release of autocooids from the tissue and basophils. The only treatment group of CGEE (400, 600 mg/kg, p.o.) able to show significant response in controlling the anaphylaxis. The marked response shown by the ethanolic extract of flowers of *Calotropis gigantea* instigates further studies to explore the mechanistic approach and component responsible for the action and valid utility of the herbs in treatment of anaphylaxis.

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